

What is claimed is:

1. A chimeric phosphorylation indicator, comprising, in operative linkage, a donor molecule, a phosphorylatable domain, a phosphoaminoacid binding domain, and an acceptor molecule,
wherein the phosphoaminoacid binding domain specifically binds to a phosphoaminoacid when present in the phosphorylatable domain,
wherein the donor molecule and the acceptor molecule exhibit a detectable resonance energy transfer when the donor is excited, and
wherein the phosphorylatable domain and phosphoaminoacid binding domain do not substantially emit light to excite the acceptor.
2. The chimeric phosphorylation indicator of claim 1, wherein at least one of the donor molecule or the acceptor molecule is a fluorescent protein.
3. The chimeric phosphorylation indicator of claim 2, wherein each of the donor molecule and the acceptor molecule is a fluorescent protein.
4. The chimeric phosphorylation indicator of claim 2, wherein the fluorescent protein is a green fluorescent protein (GFP), a red fluorescent protein (RFP), or a fluorescent protein related to a GFP or an RFP.
5. The chimeric phosphorylation indicator of claim 4, wherein the red fluorescent protein is a *Discosoma* RFP or a fluorescent protein related to a *Discosoma* RFP.
6. The chimeric phosphorylation indicator of claim 5, wherein the *Discosoma* RFP is DsRed or a mutant thereof.
7. The chimeric phosphorylation indicator of claim 6, wherein the DsRed comprises an amino acid sequence as set forth in SEQ ID NO:12 or a mutant of SEQ ID NO:12.

8. The chimeric phosphorylation indicator of claim 7, wherein the mutant of SEQ ID NO:12 comprises an I125R mutation.

9. The chimeric phosphorylation indicator of claim 4, wherein the fluorescent protein is a GFP selected from an *Aequorea* GFP, a *Renilla* GFP, a *Phialidium* GFP, or a fluorescent protein related to an *Aequorea* GFP, a *Renilla* GFP, and a *Phialidium* GFP.

10. The chimeric phosphorylation indicator of claim 9, wherein the fluorescent protein related to the *Aequorea* GFP is a cyan fluorescent protein (CFP), or a yellow fluorescent protein (YFP), or a spectral variant of the CFP or YFP.

11. The chimeric phosphorylation indicator of claim 9, wherein the fluorescent protein related to the *Aequorea* GFP is an enhanced GFP (EGFP; SEQ ID NO:4), an enhanced CFP (ECFP; SEQ ID NO:6), an ECFP(1-227) (amino acids 1 to 227 of SEQ ID NO:6), an EYFP-V68L/Q69K (SEQ ID NO:10), an enhanced YFP (EYFP; SEQ ID NO:8), or citrine.

12. The chimeric phosphorylation indicator of claim 2, wherein the fluorescent protein comprises a mutation of an amino acid residue corresponding to A206, L221, F223, or a combination thereof of SEQ ID NO:2.

13. The chimeric phosphorylation indicator of claim 12, wherein the mutation corresponds to an A206K mutation, an L221K mutation, an F223R mutation, or an L221K and F223R mutation of SEQ ID NO:2.

14. The chimeric phosphorylation indicator of claim 12, wherein the mutation corresponds to an A206K mutation, an L221K mutation, an F223R mutation, or an L221K and F223R mutation of SEQ ID NO:6 or SEQ ID NO:10.

15. The chimeric phosphorylation indicator of claim 1, wherein the donor molecule is a fluorescent protein, and the detectable resonance energy transfer is fluorescent resonance energy transfer.

16. The chimeric phosphorylation indicator of claim 1, wherein at least one of the donor molecule or the acceptor molecule is a luminescent molecule.

17. The chimeric phosphorylation indicator of claim 16, wherein the luminescent molecule comprises a lanthanide.

18. The chimeric phosphorylation indicator of claim 17, wherein the luminescent molecule comprises a terbium ion (Tb^{3+}) chelate.

19. The chimeric phosphorylation indicator of claim 18, wherein the Tb^{3+} chelate comprises Tb^{3+} and triethylenetetraaminehexaacetic acid (TTHA).

20. The chimeric phosphorylation indicator of claim 19, wherein the luminescent molecule comprises carbostyryl 124 operatively linked to the Tb^{3+} chelate.

21. The chimeric phosphorylation indicator of claim 17, wherein the luminescent molecule further comprises a cell compartmentalization domain.

22. The chimeric phosphorylation indicator of claim 21, wherein the cell compartmentalization domain is a membrane translocating domain.

23. The chimeric phosphorylation indicator of claim 22, wherein the membrane translocating domain comprises an amino acid sequence CRQIKWFNRRMKWKK (SEQ ID NO:18).

24. The chimeric phosphorylation indicator of claim 22, wherein the membrane translocating domain is operatively linked to the luminescent molecule through an amino acid sequence CCXXCC (SEQ ID NO:17).

25. The chimeric phosphorylation indicator of claim 1, wherein the donor molecule is a luminescent molecule, and the detectable resonance energy transfer is luminescent resonance energy transfer.

26. The chimeric phosphorylation indicator of claim 25, wherein the acceptor molecule is a fluorescent protein.

27. The chimeric phosphorylation indicator of claim 1, wherein the phosphorylatable domain comprises a serine/threonine kinase phosphorylatable domain.

28. The chimeric phosphorylation indicator of claim 28, wherein the serine/threonine kinase domain comprises an amino acid sequence LRRASLP (SEQ ID NO:20).

29. The chimeric phosphorylation indicator of claim 1, wherein the phosphoaminoacid binding domain comprises an amino acid sequence of 14-3-3 τ (1-232).

30. The chimeric phosphorylation indicator of claim 27, comprising, in an orientation from the amino terminus to carboxy terminus, an ECFP(1-227) (amino acids 1-227 of SEQ ID NO:6), an MH linker, a 14-3-3 τ (1-232) phosphoaminoacid binding domain, an AGGTGGS (SEQ ID NO:19) linker, an LRRASLP (SEQ ID NO:20) phosphorylatable domain, a GGTGGSEL (SEQ ID NO:21) linker, and a citrine.

31. The chimeric phosphorylation indicator of claim 1, wherein the phosphorylatable domain comprises a tyrosine kinase phosphorylatable domain.

32. The chimeric phosphorylation indicator of claim 31, wherein the tyrosine kinase phosphorylatable domain comprises an amino acid sequence selected from EEEAEYMNMAPQS (SEQ ID NO:23) and EIYGEF (SEQ ID NO:25).

33. The chimeric phosphorylation indicator of claim 1, wherein the phosphoaminoacid binding domain comprises a Src homology domain-2.

34. The chimeric phosphorylation indicator of claim 31, comprising, in an orientation from the amino terminus to carboxy terminus, an ECFP(1-227) (amino acids 1 to 227 of SEQ ID NO:6) molecule, an SH2 phosphoaminoacid binding domain from Shc, a GSHSGSGKP (SEQ ID NO:22) linker, a phosphorylatable domain comprising EEEAEYMNMAPQS (SEQ ID NO:23), and citrine.

35. The chimeric phosphorylation indicator of claim 31, comprising, in an orientation from the amino terminus to carboxy terminus, an ECFP(1-227) (amino acids 1 to 227 of SEQ ID NO:6), an SH2 phosphoaminoacid binding domain from c-src, GSTSGSGKPGSGEGS (SEQ ID NO:24), a phosphorylatable domain comprising EIYGEF (SEQ ID NO:25), and citrine.

36. The chimeric phosphorylation indicator of claim 1, wherein at least one amino acid of the phosphorylatable domain is phosphorylated.

37. The chimeric phosphorylation indicator of claim 36, wherein the amino acid is serine, threonine, tyrosine, or a combination thereof.

38. A chimeric phosphorylation indicator, comprising a phosphorylatable polypeptide and a fluorescent protein.

39. The chimeric phosphorylation indicator of claim 38, wherein the phosphorylatable polypeptide comprises an N-terminal portion and a C-terminal portion, and wherein the fluorescent protein is operatively inserted between the N-terminal portion and C-terminal portion of the phosphorylatable polypeptide.

40. The chimeric phosphorylation indicator of claim 39, wherein the fluorescent protein is a green fluorescent protein (GFP), a red fluorescent protein (RFP), or a fluorescent protein related to a GFP or an RFP.

41. The chimeric phosphorylation indicator of claim 39, wherein the fluorescent protein is a circularly permuted fluorescent protein.

42. The chimeric phosphorylation indicator of claim 39, wherein the phosphorylatable polypeptide is a substrate for a tyrosine kinase or a serine/threonine kinase.

43. The chimeric phosphorylation indicator of claim 39, wherein the fluorescent protein is operatively inserted into a hinge region or a turn in the phosphorylatable polypeptide.

44. The chimeric phosphorylation indicator of claim 38, further comprising a phosphoaminoacid binding domain operatively linked to the phosphorylatable polypeptide, wherein the fluorescent protein comprises an N-terminal portion and a C-terminal portion, and wherein the phosphorylatable polypeptide and operatively linked phosphoaminoacid binding domain is operatively inserted between the N-terminal portion and C-terminal portion of the fluorescent protein.

45. The chimeric phosphorylation indicator of claim 44, wherein the fluorescent protein is a green fluorescent protein (GFP), a red fluorescent protein (RFP), or a fluorescent protein related to a GFP or an RFP.

46. The chimeric phosphorylation indicator of claim 45, wherein the fluorescent protein is an enhanced yellow fluorescent protein (EYFP).

47. The chimeric phosphorylation indicator of claim 44, wherein the phosphorylatable polypeptide and operatively linked phosphoaminoacid binding domain is operatively inserted between an amino acid sequence corresponding to amino acid positions 145 and 146 of SEQ ID NO:2.

48. The chimeric phosphorylation indicator of claim 47, wherein the fluorescent protein is an EYFP.

49. The chimeric phosphorylation indicator of claim 38, wherein at least one amino acid of the phosphorylatable polypeptide is phosphorylated.

50. The chimeric phosphorylation indicator of claim 49, wherein the amino acid is serine, threonine, tyrosine, or a combination thereof.

51. A kit, comprising at least one chimeric phosphorylation indicator of claim 1.

52. The kit of claim 51, comprising a plurality of different chimeric phosphorylation indicators.

53. The kit of claim 52, wherein the plurality of different chimeric phosphorylation indicators comprise different phosphorylatable domains.

54. The kit of claim 52, wherein the plurality of different chimeric phosphorylation indicators comprise different donor molecule or acceptor molecules or both.

55. A kit, comprising at least one chimeric phosphorylation indicator of claim 39.

56. The kit of claim 55, comprising a plurality of different chimeric phosphorylation indicators.

57. The kit of claim 56, wherein the plurality of different chimeric phosphorylation indicators comprise different phosphorylatable polypeptides.

58. The kit of claim 56, wherein the plurality of different chimeric phosphorylation indicators comprise different fluorescent proteins.

59. A kit, comprising at least one chimeric phosphorylation indicator of claim 44.

60. The kit of claim 59, comprising a plurality of different chimeric phosphorylation indicators.

61. The kit of claim 59, wherein the plurality of different chimeric phosphorylation indicators comprise different phosphorylatable polypeptides.

62. The kit of claim 59, wherein the plurality of different chimeric phosphorylation indicators comprise different fluorescent proteins.

63. A polynucleotide encoding the chimeric phosphorylation indicator of claim 1.

64. A polynucleotide encoding the chimeric phosphorylation indicator of claim 38.

65. A vector, comprising the polynucleotide of claim 63.

66. The vector of claim 65, which is an expression vector.

67. A host cell containing the polynucleotide of claim 63.

68. A vector, comprising the polynucleotide of claim 64.

69. The vector of claim 68, which is an expression vector.

70. A host cell containing the polynucleotide of claim 64.

71. A kit, comprising at least one polynucleotide of claim 63.

72. A kit, comprising at least one polynucleotide of claim 64.

73. The polynucleotide of claim 63, which is operatively linked to an expression control sequence.

74. The polynucleotide of claim 73, wherein the expression control sequence is a transcription regulatory element, a translation regulatory element, or a combination thereof.

75. The polynucleotide of claim 64, which is operatively linked to an expression control sequence.

76. The polynucleotide of claim 75, wherein the expression control sequence is a transcription regulatory element, a translation regulatory element, or a combination thereof.

77. A method for detecting a kinase or phosphatase in a sample, the method comprising:

contacting the sample with a chimeric phosphorylatable indicator of claim 1,

exciting the donor molecule; and

determining a fluorescence or luminescence property in the sample,

wherein the presence of a kinase or phosphatase in the sample results in a change in the degree of fluorescence resonance energy transfer (FRET) or luminescence resonance energy transfer (LRET), thereby detecting the kinase or phosphatase in the sample.

78. The method of claim 77, wherein the change in the degree of FRET or LRET is an increased amount of FRET or LRET.

79. The method of claim 77, wherein the change in the degree of FRET or LRET is a decreased amount of FRET or LRET.

80. The method of claim 77, wherein a change in the degree of FRET or LRET is indicative of a kinase in the sample.

81. The method of claim 77, wherein the phosphorylatable domain is phosphorylated prior to contacting the sample with a chimeric phosphorylatable indicator.

82. The method of claim 81, wherein a change in the degree of FRET or LRET is indicative of a phosphatase in the sample.

83. The method of claim 77, wherein the sample is a biological sample.

84. The method of claim 83, wherein the biological sample comprises a cell, a tissue sample, or an extract of a cell or a tissue sample.

85. The method of claim 77, wherein said detecting is performed on an intact cell or tissue sample.

86. The method of claim 85, wherein the chimeric phosphorylatable indicator further comprises a targeting sequence.

87. The method of claim 86, wherein the targeting sequence comprises a cell compartmentalization domain.

88. The method of claim 87, wherein the cell compartmentalization domain targets the chimeric phosphorylatable indicator to cytosol, endoplasmic reticulum, mitochondrial matrix, chloroplast lumen, medial trans-Golgi cisternae, a lumen of a lysosome, or a lumen of an endosome.

89. The method of claim 86, wherein the cell compartmentalization domain is a membrane translocating domain.

90. A method for detecting a kinase or phosphatase in a sample, the method comprising:

contacting the sample with a chimeric phosphorylatable indicator of claim 38,

determining a fluorescence property in the sample,

wherein the presence of kinase or phosphatase activity in the sample results in a change in the fluorescence property as compared to the fluorescent property in the absence of a kinase or phosphatase activity, thereby detecting the kinase or phosphatase in the sample.

91. The method of claim 90, wherein the absence of kinase or phosphatase activity in the sample is due to the presence of a kinase inhibitor or phosphatase inhibitor.

92. The method of claim 91, wherein the kinase inhibitor is a serine/threonine kinase inhibitor or a tyrosine kinase inhibitor.

93. A method for detecting a kinase inhibitor or phosphatase inhibitor, the method comprising:

determining a first fluorescence property of a chimeric phosphorylatable indicator of claim 1 in the presence of a kinase or a phosphatase,

contacting the chimeric phosphorylatable indicator with a composition suspected of being a kinase inhibitor or a phosphatase inhibitor,

determining a second fluorescence property of a chimeric phosphorylatable indicator in the presence of the composition, wherein a difference in the first fluorescence property and second fluorescence property identifies the composition as a kinase inhibitor or phosphatase inhibitor.

94. The method of claim 93, which is adapted to high throughput analysis.

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